TABLE 3
T2 Seeds having Decreased
C18:2 (WS201[1] Transformants)[2].

Line No.	Plant Series	Fatty Acid Composition (%) C16:0	C18:0	C18:1	C18:2	C18:3
T300236	WS201	4.1	3.2	72.5	10.6	6.5
T300238	WS201	3.9	2.7	74.4	9.2	6.6
T300390	WS201	4.2	2.5	75.4	9.3	5.4
T300273	WS201	3.9	2.8	77.4	7.5	5.3
T300400	WS201	3.9	2.5	78.6	6.6	5.3
T300354	WS201	4.0	2.7	78.6	6.4	4.51

[1] WS201: phaseolin promoter/IMC129 mutant, sense orientation.
[2] Population of 168 selfed individuals from greenhouse.

As shown in Figure 3, a large proportion of the control WS127 plants have elevated levels of linoleic acid. This result is expected, since the delta-12 desaturase gene dosage is higher due to the extra copy of the wild-type delta-12 desaturase gene. A small proportion of WS127 plants show lower levels of linoleic acid, due to cosuppression.

In contrast, no WS201 plants had elevated levels of linoleic acid. This result confirms that the mutant delta-12 desaturase D form gene in WS201 plants is non-functional. Furthermore, a higher proportion of WS201 plants have decreased C18:2 (5-14%) compared to the proportion of WS127 control plants having decreased C18:2. The proportion of WS201 plants having decreased C18:2 is about 25-fold higher than the proportion of WS127 plants having decreased C18:2. The surprising inhibition of endogenous delta-12 desaturase activity by a mutant delta-12 gene product has been termed dominant negative suppression.

The fatty acid composition of seeds produced by representative dominant negative suppression T2 WS201 plants is shown in Table 6. The plants show an altered fatty acid composition, including decreased C18:2, increased C18:1, and decreased saturates.

A T2 generation plant is not homozygous for the introduc d gene. Consequently, T3 and subsequent generations that are homozygous for the mutant delta-12 desaturase gene will have even lower levels of linoleic acid, from about 1% to about 10%, preferably from about 1% to about 6%. Levels of oleic acid in homozygous lines will be from 75% to about 88%, preferably from about 80% to about 88%.

The results observed with WS140 plants (containing a mutant Q508 F form delta-12 desaturase gene) are shown in Figure 4. None of the WS140 plants have elevated C18:2 levels, similar to the results obtained with WS201 transgenic plants. As expected, a large proportion of the control WS135 plants have elevated C18:2 levels. The proportion of WS140 and WS135 plants having decreased C18:2 levels is similar, indicating that expression of this particular mutant delta-12 desaturase gene product does not inhibit endogenous wild-type delta-12 desaturase gene product to an extent greater than that expected from cosuppression. The fatty acid composition of seeds produced by a representative T2 WS140 plant with decreased C18:2 levels is shown in Table 4.

TABLE 4

T2 Seed Having Decreased C18:2 (WS140[1] Transformants)[2].

		Fatty Acid Composition (%)				
Line No.	Vector	C16:0	C18:0	C18:1	C18:2	C18:3
T300435	pIMC140	3.8	3.5	73.7	9.7	6.11

[1] WS140: napin promoter/Q508 fad2 F mutation, sense orientation

[2] Population of 61 selfed individuals from greenhouse

Brassica Shoot Elongation Medium MSV-1A

Murashige and Skoog Minimal Organic M dium
Gamborg B5 Vitamins
10 grams sucrose
0.6% agarose
pH 5.8

Once shoots had elongated several internodes, they were cut above the agar surface and the cut ends were dipped in Rootone. Treated shoots were planted directly into wet Metro-Mix 350 soiless potting medium. The pots were covered with plastic bags which were removed when the plants were clearly growing -- after about ten days.

Plants were grown under a 16:8 h photoperiod, with a daytime temperature of 23°C and a nightime temperature of 17°C. When the primary flowering stem began to elongate, it was covered with a mesh pollen-containment bag to prevent outcrossing. Self-pollination was facilitated by shaking the plants several times each day.

Transgenic progeny plants containing pZPhMCFd2 were designated as the WS201 series. Plants transformed with pIMC127 were designated as the WS127 series. Plants transformed with pIMC135 were designated as the WS135 series. Plants transformed with pIMC140 were designated as the WS140 series. Seeds were obtained by selfing the T1 plants. Fatty acid profiles of the T2 seeds were determined as described in WO 93/11245. The results are shown in Tables 6 and 7 and Figures 3 and 4.